*p*H 13, the carboxyl group is not split off during the condensation. In a subsequent note we studied other cases of condensation of aldehydes with β-oxo acids with liberation of carbon dioxide within the *p*H range 3–11 at room temperature,[3] and extended our research to the condensation of aldehyde ammonias with β-oxo acids.[4] We, therefore, were the first to observe that the products of the reactions in question depend on the *p*H, and that in this instance within an approximately physiological *p*H-range the aldol condensation is coupled with the liberation of carbon dioxide, a fact which Henze[5] failed to notice in his study on the condensation of methylglyoxal with acetoacetic acid.

The condensation of methylglyoxal with β-oxo acids represents a special case of these "syntheses under physiological conditions." According to our former results it was to be expected that the condensation studied by Schechter, Green and LaForge should occur in the examined *p*H-range with spontaneous decarboxylation.

(3) Schöpf and Thierfelder, *Ann.*, **518**, 127 (1935).

(4) Schöpf and Lehmann, *Ann.*, **518**, 1 (1935); *cf. Angew. Chem.*, **50**, 779, 797 (1937), summary, with special reference to p. 783.

(5) Henze, *Z. physiol. Chem.*, **189**, 121 (1930). According to *ibid.*, **193**, 88 (1930), and **200**, 104 (1931), the carboxylic acid referred to above is described as being the first product of reaction; *Z. physiol. Chem.*, **214**, 281 (1933), subsequent heating to 50° has been prescribed to speed liberation of carbon dioxide.

INSTITUT FÜR ORGANISCHE CHEMIE
DER TECHNISCHEN HOCHSCHULE DARMSTADT
DARMSTADT, GERMANY CLEMENS SCHÖPF

RECEIVED MAY 4, 1950

SPECIFICITY OF UREASE ACTION: A CORRECTION

Sir:

In a letter under the above title[1] we reported partial hydrolysis of commercial C. P. biuret by the enzyme urease. Puzzled by the incompleteness of this reaction we have continued its study and have reached a definite conclusion that biuret is not hydrolyzed by urease. We were misled by the assurance of the manufacturer that the biuret was C. P. and also by the excellent agreement of elementary analysis with the calculated composition. Thus material as received gave N, 34.86, biuret monohydrate being calculated to give 34.7. After drying at 115°, as recommended, the loss in weight was 14.8%, 14.9% being calculated for the monohydrate. The residue analyzed as follows: N, 40.8 (40.8); C, 23.5 (23.3); H, 4.65 (4.85), the parenthesized figures referring to calculation for biuret. However, a later analysis of material which was dried in high vacuum at 40° gave N, 37.3; C, 23.4; H, 4.77, which does not agree with that of any mixture of biuret and its monohydrate.

It has been now established that the composition is apparently only accidentally that of biuret and that the material is a rather complex mixture of several compounds. In particular, it contains about 10% of total nitrogen which is hydrolyzed by urease[2] and which is contained in a compound that is more soluble in water than is biuret. Extraction with water suggests that this material is present in biuret as a solid solution rather than as separate crystals. After tedious fractional crystallizations from alcohol a material has been repeatedly obtained with a rather sharp melting point at 110 to 115°, which is somewhat lowered by additions of urea. Analysis of a typical preparation gave N, 44.6; C, 21.2; H, 6.2, but the composition appears to vary slightly with the method of purification. Its molecular weight, determined by the freezing point lowering method in water, ranges from 70 to 85. Total hydrolysis by urease gives nitrogen content of 33 to 41%. Infrared spectrum, obtained in Nujol suspension, is indistinguishable from that of urea. Although some of the above data are inconsistent with this conclusion, the most probable interpretation of the data is that the isolated substance is not a pure chemical compound but is a loose compound or solid solution of urea and something else with a similar elementary composition. Therefore our results to date do not prove the existence of another substrate for urease besides urea.

(1) THIS JOURNAL, **72**, 634 (1950).

(2) Hydrolysis of 33% of total biuret nitrogen reported in the previous communication was obtained on making the solution with excess biuret and was evidently due to the preferential solubility of the hydrolyzable compound.

DEPARTMENT OF CHEMISTRY
HARVARD UNIVERSITY W. H. R. SHAW
CAMBRIDGE, MASS. G. B. KISTIAKOWSKY

RECEIVED MAY 1, 1950

SYNTHETIC MEMBERS OF THE FOLINIC ACID GROUP

Sir:

In attempts to synthesize certain derivatives of folic acid which might possess activity in replacing folinic acid for *Lactobacillus casei*[1] and for *Leuconostoc citrovorum* 8081,[2,3] we have found that highly active material can be produced from folic acid as follows: Folic acid (500 mg.) is treated with sufficient (approximately 5 cc.) formic acid (98%) containing 20% acetic anhydride to effect solution of the folic acid. The reaction mixture is heated for one hour at 50°, and the resulting crude formylfolic acid is obtained by evaporation of the excess reagent in a frozen state under reduced pressure. The crude formylfolic acid and 2 g. of ascorbic acid are dissolved in 50 cc. of water, and the *p*H is adjusted with sodium carbonate to 7.2–7.6. The mixture is hydrogenated in the presence of platinum oxide as a catalyst until approximately 1 mole of hydrogen per mole of folic acid is consumed. After hydrogenation, the material is not highly active in the assays, but after autoclaving the reaction mixture at 120° for one hour, an amount of the reaction mixture equivalent to 0.00004 to 0.00001 γ of the original folic acid per

(1) Bond, *et al.*, THIS JOURNAL, **71**, 3852 (1949).

(2) Sauberlich and Baumann, *J. Biol. Chem.*, **176**, 165 (1948).

(3) Bardos, *et al.*, THIS JOURNAL, **71**, 3852 (1949).